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Nutritional Needs In Environmental Intoxication: Vitamin E and Air Pollution, An Example

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Dietary vitamin E affects the susceptibility of mice and rats to ozone and nitrogen dioxide, suggesting a free radical mechanism of toxicity. Conventional peroxidation does not completely explain the effects of alterations of lung fatty acid composition on both nitrogen dioxide and ozone toxicity. A new scheme is proposed based on the cyclization of β, γ-allylic peroxyl free radicals to monocyclic and bicyclic peroxides to explain the relationship between diet and toxicity. Similar results are likely with other toxicants producing peroxidation as a mechanism of toxicity. Such cyclic peroxides may mimic or interfere with the prostaglandin system. Several chronic diseases may be exacerbated through such a subtle toxic mechanism. The level of vitamin E needed for protection against peroxidation toxicity may be much greater than the present U. S. dietary intake.

Introduction

Prevention of disease is the primary purpose of environmental medicine. This symposium seeks to identify those segments of the human population especially at risk to toxicity from environmental toxicants because of genetic, social, or economic factors. In this discussion, the impact of nutrition will be considered using the effects of oxidizing air pollutants on the lung as an example. The toxicities of ozone (O_3) and nitrogen dioxide (NO_2) are particularly useful examples, since the response of the lung to these toxicants can be easily modified by dietary control. The question of dietary impact on the toxicity of environmental toxicants is not restricted to the air pollutants, however. In man, diet can affect metabolism of drugs, carcinogenicity of organic compounds, maximum growth, stature, mental acuity, reproductive capacity, hormonal elaboration, and susceptibility to infection. Improper nutrition has commonly led to obesity, which is a major contributor to cardiovascular disease, hypertension, and adult-onset diabetes. Diet, then, is one of the most important variables available for prevention of disease.

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Oxidizing Air Pollutants as Initiators of Free-Radical Reactions

Our basic hypothesis has been that oxidizing air pollutants produce their toxicity through free radical reactions involving unsaturated fatty acids. Most unsaturated fatty acids are esterified to phospholipids which, in turn, are present in the membranes of cells. Free unsaturated fatty acids, especially arachidonic acid (C 20:4 ω6) and eicosatrienoic acid (C 20:3 ω6), the precursors of the prostaglandins, exist in relatively low concentrations in the circulation. Both NO_2 and O_3 can react with unsaturated fatty acids to produce peroxides. NO_2 can react via the scheme shown in Eqs. (1-(5).

$$RCH = CHR + NO_2 \rightarrow R\dot{C}HCHNO_2R' \quad (1)$$

$$R\dot{C}HCHNO_2R' + O_2 \rightarrow R\text{-}\underset{\displaystyle O_2\cdot}{\underset{|}{C}}HCHNO_2R' \quad (2)$$

$$R''H + \underset{\displaystyle O_2\cdot}{\underset{|}{R\dot{C}}}HCHNO_2R' \rightarrow R'''\cdot + \underset{\displaystyle O_2H}{\underset{|}{RC}}HCHNO_2R' \quad (3)$$

$$R'''\cdot + O_2 \rightarrow R'''O_2\cdot \quad (4)$$

$$R''O_2\cdot + RH \rightarrow R''O_2H + R\cdot \quad (5)$$

Alternatively (Pryor, personal communication), NO_2 can abstract a hydrogen directly to form an alkyl free radical which can react with molecular oxygen as in reaction (4).

$$RH + ONO \longrightarrow R\cdot + HONO \quad (6)$$

$$R\cdot + O_2 \longrightarrow RO_2\cdot \quad (7)$$

O_3 catalyzes peroxidation of unsaturated fatty acids by direct ionic addition across the ethylene group as proposed by Criegee [Eqs. (8)-(11)].

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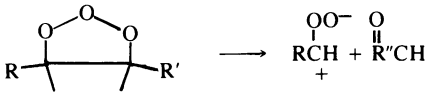

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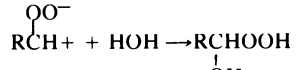

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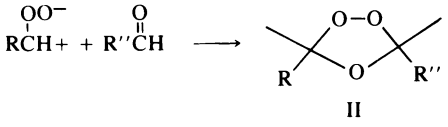

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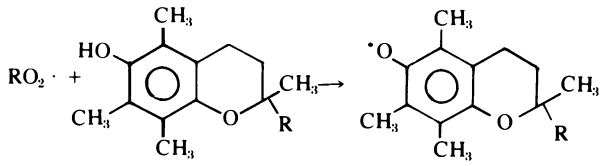
The hydroxy hydroperoxide I could decompose to peroxyl radicals and initiate peroxidation via the autoxidation mechanism of reactions (4) and (5). The fatty acid ozonide II can initiate peroxidation of isolated microsomes, mitochondria, and nuclei (Menzel, unpublished observations). The reaction consumes more oxygen than the equivalent amount of ozonide added to the reaction mixture and is inhibited by the addition of phenolic antioxidants. Boiled microsomes or microsomes digested with trypsin function almost as well in the reaction, suggesting that it is not enzymatic. Injection of fatty acid ozonides produces pulmonary edema (*1*) and Heinz body formation (*2*).

Both the NO_2- and O_3-catalyzed peroxidations of unsaturated fatty acids are inhibited by phenolic antioxidants including vitamin E. Vitamin E reacts with the hydroperoxyl radical to form the corresponding hydroperoxide and a stable tocopherol free radical. The tocopherol-free radicals dimerize easily and a metabolite of the tocopherol dimer is the principal urinary excretory metabolite of vitamin E in animals.



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Effects of Vitamin E on the Toxicity of Ozone and Nitrogen Dioxide

Vitamin E is a poor scavenger of both O_3 and NO_2. As many as six products can be isolated from α-tocopherol exposed to ozone, but the rate of the reaction is slower than the peroxidation of unsaturated fatty acids. Vitamin E acts as an antioxidant to terminate the peroxidation initiated by the free-radical mechanism, e.g., termination of reactions such as reaction (5). Depriving rats or mice of vitamin E increases the toxicity of both NO_2 and O_3. Rats fed vitamin E-free diets are significantly more susceptible to both NO_2 and O_3 toxicity (*3–5*). The addition of other antioxidants, selenium or ascorbic acid, to the diet of rats exposed to high levels of O_3 (above 2 ppm) does not afford additional protection (*4*). Generally, these experiments were undertaken by using diets supplemented with 100 mg vitamin E acetate/kg of diet. The average U. S. adult intake of mixed tocopherols amounts to about 17 I. U. of vitamin E. Since the amount of food ingested by rats fed the vitamin E-fortified diets varied, it is not possible to calculate an exact intake. Most rats would have eaten about 1.5 I.U. vitamin E/kg body weight/day. For an adult male, the average human consumption would correspond to about 2.4 I.U./kg body weight/day, or about two times the level ingested by rats on the 100 mg vitamin E/kg diet. Some individuals may have much lower intakes of vitamin E, since most of the vitamin E in unfortified human diets comes from fresh vegetables, salad oil, and nuts. The daily consumption of a salad is, unfortunately, an upper middle class dietary habit hardly ever adhered to by lower socioeconomic groups.

To extend these observations closer to human dietary experience, mice were fed chemically defined diets as set out in Table 1. The source of dietary fat was either stripped corn oil or lard. These fats had been rendered free of all but very minute amounts of vitamin E by vacuum distillation. In the absence of fortification, the diets are essentially free

Table 1. Composition of chemically defined diets fed to mice exposed to NO_2 or O_3.

Component	Weight %
Casein, vitamin-free[a]	22.8
Williams-Briggs modified mineral mix[a]	3.5
Sucrose	68.1
Fats (stripped corn oil or lard)[b]	5.0
Choline dihydrogen citrate	0.3
Vitamin A[c]	24,000 IU/kg
Vitamin D_3	2,400 IU/kg
Vitamin mix[d]	0.3
Sodium selenate	10 μg/100 g

[a] Teklad Test Diets, Chagrin Falls, Ohio.
[b] Eastman Organic Chemicals, Rochester, New York.
[c] Administered as retinyl palmitate.
[d] Vitamin mix content (mg/0.3 g): inositol, 11.1; *p*-aminobenzoic acid, 11.1; *D*-calcium pantothenate, 6.2; 2-methyl-1,4-naphthoquinone, 5.0; niacin, 10.0; thiamin · HCl, 2.2; pyridoxine · HCl, 2.2; riboflavin, 2.2; folic acid, 0.2; biotin, 45 μg; vitamin B_{12}, 3 μg; sucrose to 0.3 g.

of vitamin E. Mice were fed these diets for 6 weeks in order to equilibrate the lungs to the fatty acid composition and vitamin E content of the diets. There were no differences in growth between groups. (Fig. 1) The fatty acid composition of the lungs of these animals is set out in Tables 2 and 3. The peroxidizability index, which is a weighted average based on the relative rates of peroxidation of single purified fatty acids, was calculated according to the formula shown in Table 2. The distribution of fatty acids is also reported in terms of the saturated, mono-, di-, and polyenoic fatty acids (Table 3). One can see that it was possible to manipulate the fatty acid composition independent of the vitamin E content. The lard-fed groups consisted of mice having lungs of a more saturated fatty acid composition, while the corn oil-fed groups consisted of those having a more unsaturated fatty acid composition.

The mortality of these mice on continuous exposure to 0.97 ± 0.03 ppm O_3 is shown in Figures 2 and 3 (*6*). The LT_{50} for mice fed either no or 10.5 mg vitamin E/kg was 29 days for the corn oil-fed group and 32 days for the lard-fed groups. In contrast the groups receiving 105 mg vitamin E acetate/kg had an LT_{50} of 44 days for the lard-fed group and 47 days for the corn oil-fed groups. In other words, fortification with 100 mg vitamin E/kg provided increased longevity by 38% in the lard-fed mice and by 62% in the corn oil-fed mice. These diets are relatively low in fat, having only 11% of calories as either lard or corn oil. The U. S. diet varies from 35 to 45% of calories as fat. Increasing the fat content to 30–40% of the caloric intake could exacerbate the effects of low or no vitamin E intake. Experiments are in progress to test this point.

Nitrogen dioxide is less toxic than O_3, though a demonstrated health hazard to man at environmental levels. Mortality studies have been undertaken at 33 ppm or greater (*3–5*). The effect of lower concentrations of NO_2 is less marked and generally results in pulmonary edema and eventually emphysema. A crude quantitative index of interstitial

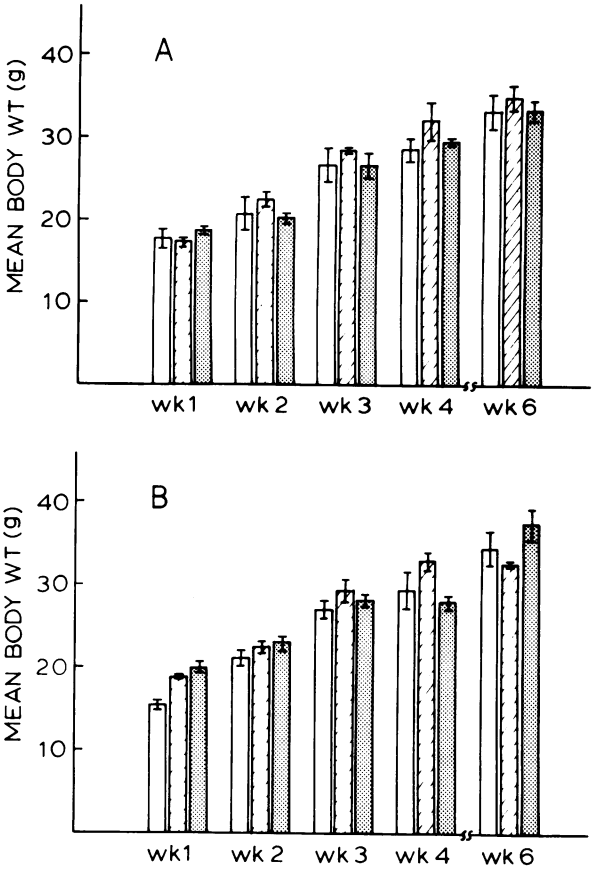


FIGURE 1. Mean body weights of (A) lard-fed and (B) corn oil-fed mice: (□) 0 vitamin E; (▨) 10.5 mg vitamin E acetate/kg diet; (□) 105 mg vitamin E acetate/kg diet. Each value is the mean ± SE of five mice.

Table 2. Calculated peroxidizability index (PI) of mouse lung total fatty acids at 6 weeks.

Supplemental vitamin E acetate, mg/kg	Peroxidizability index[a]	
	Lard-fed groups	Corn oil-fed groups
0	57.51 ± 3.68	78.87 ± 3.10
10.5	59.71 ± 1.01	72.83 ± 1.16
105	58.71 ± 1.81	69.72 ± 0.68

[a] Presented as mean ± SEM of five animals per diet group. The index was calculated according to the formula: PI = (% monoenoic × 0.025) + (% dienoic × 1) + (% trienoic × 2) + (% tetraenoic × 4) + (% pentaenoic × 6) + (% hexaenoic × 8).

Table 3. Distribution of unsaturated, monoenoic, dienoic, and polyenoic fatty acids among mouse lung total fatty acids of mice fed supplemental vitamin E.[a]

Fatty acid class	Lard-fed groups			Corn oil-fed groups		
	0 Vitamin E	10.5 mg/kg	105 mg/kg	0 Vitamin E	10.5 mg/kg	105 mg/kg
Saturate	48.26 ± 0.47	47.86 ± 0.45	48.32 ± 0.40	48.82 ± 0.56	48.67 ± 0.38	47.84 ± 0.35
Monoenoic	35.20 ± 0.62	35.34 ± 0.58	34.94 ± 0.71	28.52 ± 0.64	28.41 ± 0.48	28.17 ± 0.69
Dienoic	3.88 ± 0.07	3.81 ± 0.09	3.85 ± 0.13	7.93 ± 0.22	7.84 ± 0.14	8.68 ± 0.18
Polyenoic	12.72 ± 0.33	12.52 ± 0.25	12.74 ± 0.36	15.18 ± 0.33	15.09 ± 0.27	15.30 ± 0.35

[a] Mean weight % ± SEM of 15 mice per diet group for week 6.

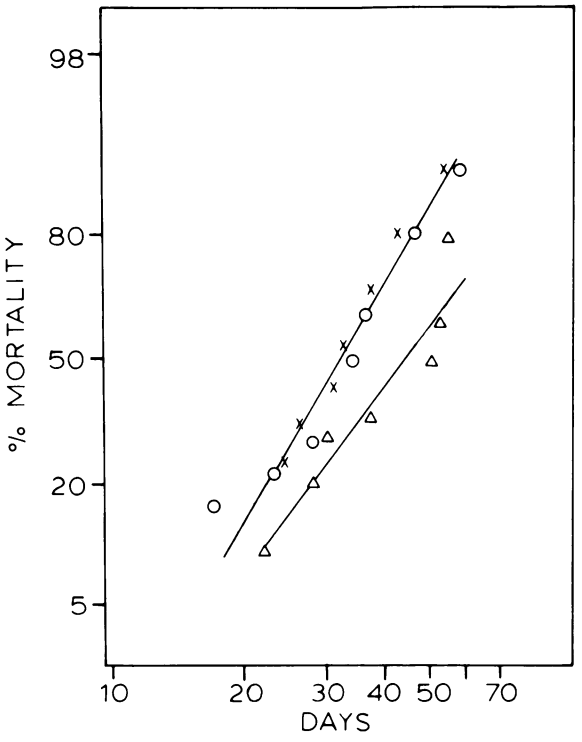
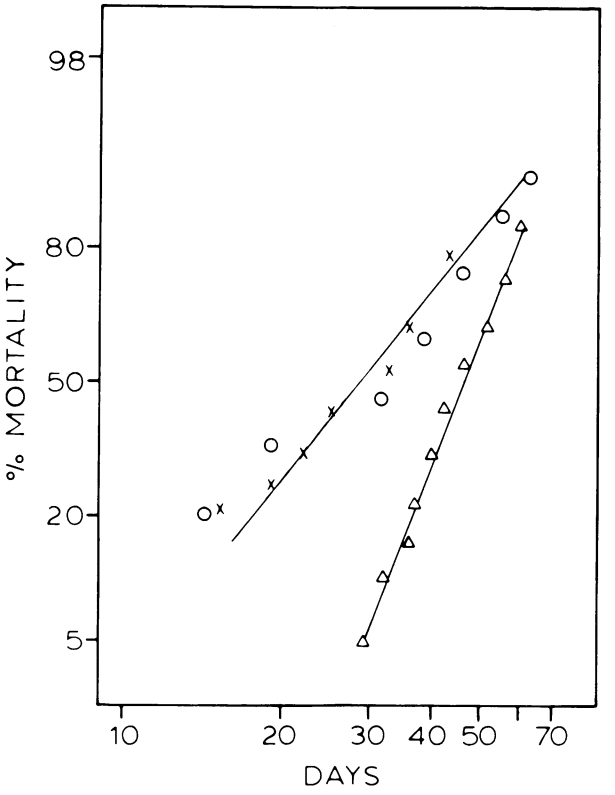


FIGURE 2. Mortality of mice exposed continuously to 0.97 ± 0.03 ppm ozone and fed diets containing 5% stripped lard which were supplemented with (○), 0 mg, (X) 10.5 mg, and (△) 105.0 mg vitamin E acetate/kg diet. Data from Donovan et al. (6) with permission.



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edema is wet weight. When mice fed these diets were exposed to 13.0 ± 0.2 ppm NO_2 for 8 hr/day for 90 days, no mortality was observed, but a significant increase in the lung weights of the exposed groups occurred (Table 4). There was a trend toward less edema in the groups receiving 10 or 100 mg vitamin E/kg of diet, but the differences in mean weights were only marginally significant due mainly to the large variance in the vitamin E deficient groups.

Exposure to O_3 also increased the relative percent of the total lung fatty acids present as arachidonic acid as shown in Table 5. A similar increase was also found in the lungs of mice exposed to NO_2 (Table 6). In the latter measurements, the total pool of arachidonic acid in each lung was determined by the use of an internal standard of C 17:0 fatty acid. The results are reported as μg of arachidonic acid/lung illustrating an increase of about 40% in the arachidonic acid pool on exposure to NO_2. Considering the low levels of arachidonic acid normally found in the lung, the increased level is of major biological significance. Since arachidonic acid is the precursor for prostaglandins, the increased pool may represent an increased demand for prostaglandins during the edema and inflammation produced by NO_2 exposure.

Table 4. Effect of dietary vitamin E on lung edema of mice exposed to 13.0 ± 0.2 ppm NO_2 for 8 hr/day for 90 days.

Group	Lung wet weight, mg[a]		
	0 Vitamin E	10.5 mg/kg	105 mg/kg
Saturated fat			
NO_2 exposed	361.3 ± 50.3[b]	242.6 ± 12.9[b]	257.5 ± 18.0[b]
Air exposed	181.9 ± 4.9	191.5 ± 6.4	187.5 ± 3.9
Unsaturated fat			
NO_2 exposed	364.5 ± 92.6[c]	236.6 ± 12.4[b]	230.1 ± 11.3[b]
Air exposed	191.5 ± 5.9	162.6 ± 14.6	179.1 ± 2.7

[a] Mean ± SE of five mice in each group.
[b] Lung weights of exposed mice were significantly elevated, $p < 0.005$.
[c] Lung weights of exposed mice were significantly elevated, $p < 0.025$.

Table 5. Alteration of lung levels of arachidonic acid in mice exposed to 0.84 ppm ozone for 4 weeks.

Group	Methyl arachidonate, weight %		
	0 Vitamin E	10.5 mg/kg	105 mg/kg
Saturated fat			
O_3 Exposed	7.49 ± 0.18	8.35 ± 0.05	7.80 ± 0.34
Air Exposed	8.01 ± 0.28	7.40 ± 0.47	7.81 ± 0.03
Unsaturated fat			
O_3 Exposed	8.99 ± 0.20[a]	9.48 ± 0.43	9.18 ± 0.13[a]
Air Exposed	7.58 ± 0.45	8.53 ± 0.12	8.25 ± 0.32

[a] $p < 0.05$.

Table 6. Elevation of the lung arachidonic acid pool in mice exposed to 13.0 ± 0.2 ppm NO_2 for 8 hr/day for 90 days.

Group	Arachidonic acid, μg per lung		
	Air exposed	NO_2 exposed	Control
Vitamin E-deficient	78.41 ± 4.88	107.24 ± 14.94	137
Vitamin E-supplemented (100 mg/kg Diet)	56.41 ± 2.43	83.79 ± 6.29[a]	149

[a] $p < 0.01$.

Hypothesis for the Biological Action of Peroxides Formed by Ozone and Nitrogen Dioxide

Interestingly, the LT_{50} values for mice whose lungs were either of a saturated or unsaturated fatty acid composition were the same in the absence or presence of vitamin E (Figs. 2 and 3). Earlier mortality was observed in the saturated fat (lard-fed) groups than in the unsaturated fat (corn oil-fed) groups. The delay in mortality was especially marked in the corn oil-fed group receiving 100 mg vitamin E/kg where no mortality was observed before 30 days, contrasted to 20% mortality in the corresponding lard-fed group. The peroxidizability index (PI) of the corn oil-fed groups, as shown in Table 2, was significantly greater than that of the lard-fed groups, suggesting a greater predicted rate of peroxidation of the lung fatty acids. Yet the LT_{50} depended upon the vitamin E content and not the fatty acid content.

Polyunsaturated fatty acids (PUFA) autoxidize following hydrogen abstraction of an allylic hydrogen:

$$RH + I\cdot \longrightarrow R\cdot \qquad (13)$$

Reactions (4) and (5) propagate the autoxidation and result in hydroperoxides as the end products. Termination of the reaction could also involve the dimerization of two alkyl radicals, but this does not appear to occur in biological systems where reaction with an antioxidant, vitamin E, appears to be the dominant chain termination step. In the *in vitro* autoxidation of PUFA, hydroperoxide formation [reaction (5)] is the rate-limiting step. The rate also depends upon the chemical structure of the fatty acid. Polyenoic fatty acids autoxidize at a disproportionate rate compared to monoenoic fatty acids. The initiation reaction, [reaction (13)] and the scavenging of alkyl radical by oxygen [reaction (5)] are rapid. If the LT_{50} of O_3 exposure were dependent on the PI, then hydroperoxide formation would appear rate-limiting, since this propagation step is dependent on the chemical nature and the abundance of the hydrogen donor. As the LT_{50} values were similar for both high and low PI groups, hydroperoxide formation was apparently not rate-limiting *in vivo*.

Given that tissue PO_2 is substantially lower than the one atmosphere at which the rate equations for autoxidation were determined *in vitro*, peroxyl radical formation [reaction (4)] may become rate-limiting in tissue autoxidation. But the initiation reaction [reaction (13)] is most likely rate-limiting on O_3 exposure, since the LT_{50} depends on the concentration of O_3. When mice were exposed to 1.6 ppm O_3, for example, a 30-fold decrease in the LT_{50} was observed compared to that at 0.97 ppm.

Vitamin E afforded protection against O_3 at only the 100 mg vitamin E/kg level, suggesting the idea that termination by reaction 13 did not become significant *in vivo* below that level. The rate of initiation of peroxidation is rate limiting under those conditions. The protective interaction of unsaturated fatty acid diets and vitamin E emphasizes the complexity of the multivariate biological situation. Under ambient conditions, the absorption and storage interactions of vitamin E and PUFA do not pre-

sent a simple, linear relationship (*7*). Ozone exposure further complicated the relationship by depleting vitamin E stores (*3, 8*), and in the rat and mouse by altering the composition of lung PUFA, notably increasing the level of arachidonic acid (*9*). The latter effect is paradoxical in terms of traditional concepts of autoxidation, and a companion effect unexplained by traditional concepts was the protection afforded by high tissue PUFA and vitamin E. The requisite level of lipid antioxidant in tissue, expressed in the vitamin E/PUFA ratio, has a critical threshold below which deficiency symptoms appear and oxidant susceptibility is enhanced (7, 10). Mice fed the lard diet containing 100 mg vitamin E/kg diet and possessing the highest vitamin E/PUFA ratio would have been expected to show the longest period before mortality.

The failure of the mortality of different diet groups to correlate with lung PI brings into question the significance of differences in PI in the lungs of the corn oil- and lard-fed groups. The difference of 22.4% in PI between the groups after 6 weeks is similar to that reported for RBC phospholipids (*7*), where hydrogen peroxide hemolysis showed a correlation with PI. An increase of 18.5% in the PI of rat muscle phospholipids was also found to correlate with the onset of creatinuria with vitamin E deficiency (*11*). However, trienoic acid diets produced creatinuria within 12 weeks while dienoic acid diets required 19 weeks.

The lung receives the brunt of oxidant exposure, and given the reactivity of O_3, little of the gas would be expected to pass the pulmonary air-blood barrier. Rather, the primary toxic effects of O_3, and probably NO_2, are exerted in the lung, and O_3 mortality is usually attributed to pulmonary edema. Similarly, pulmonary edema is the precursor of emphysema induced by NO_2 exposure. The PI of the lung was found to be significantly elevated by dietary manipulation but was not rate-limiting in the induction of mortality to O_3 or in the extent of pulmonary damage to NO_2. For a complex organ such as the lung, one may argue that mechanisms of respiratory control, defense, and morphological diversity render the PI useless. The argument raises the possibility that the PI is an inadequate measure of oxidant susceptibility in other organs as well.

Alternative Interpretation of the Toxicity of Fatty Acid Peroxidation

It is clear that a simple interpretation of O_3 or NO_2 toxicity based on the peroxidation of unsaturated fatty acids is an oversimplification. As a free radical initiator, O_3 could generate sufficient hydroperoxides and other oxidation products to cause membrane disruption and consequent cellular damage (*12*). In support of direct membrane damage, both NO_2 and O_3 exposure *in vivo* produce pulmonary macrophages which are more easily agglutinated but contain surface membrane binding sites of the same number and avidity for the ligand (*13*). The direct peroxidation damage, however, would be quantitatively dependent upon both the PI and the concentration of O_3 and NO_2. A second mechanism for oxidant toxicity could be through the generation of specific fatty acid oxidation products possessing biological activity through their potential for mimicking or antagonizing the activity of endogenous compounds. Similarities exist between the fatty acid peroxides and the naturally occurring fatty acid peroxides of the prostaglandin system. Such biopotent compounds could be generated in picogram quantities and yet be sufficiently active to disrupt pulmonary vascular regulation which is dependent upon the prostaglandin system.

The toxic products could be formed on the direct reaction with O_3 or NO_2 or by subsequent peroxidation steps. The peroxides formed by these means differ from the natural products in stereochemistry, specificity of substitution of peroxidic groups, and the potential existence of peroxidic esters. The natural prostaglandin synthesis is regulated by the requirement of cyclo-oxygenase for an unesterified carboxylic acid group. Arachidonic acid, the preferred substrate, is not available for oxygenation until hydrolyzed from phospholipids by phospholipase. Human platelets contain about 50% arachidonic acid in their phospholipids and are aggregated by conversion of the endogenous arachidonic acid to thromboxane A_2 only by stimuli which result in activation of phospholipase. In the proposed alternative explanation of peroxidation toxicity, biopotent peroxides would be formed only from fatty acids having three or more double bonds since they are most likely to yield endoperoxides through the unimolecular cyclization of a peroxyl radical (*14*).

The reaction sequence leading to cyclic peroxides of fatty acids is shown in Figure 4. Abstraction of an allylic hydrogen of a trienoic fatty acid results in an alkyl free radical having several resonance forms. Conventional autoxidation could take place with the formation of hydroperoxides containing conjugated and nonconjugated ethylene groups. Reaction with molecular oxygen to form a β,γ-allylic peroxyl radical allows a unimolecular reaction to form a cyclic peroxide. Cyclization reactions have been shown to occur using peroxyl precursors, and endoperoxide formation has been proposed to be a source of fatty acid prostaglandins, formed by O_3-initiated peroxi-

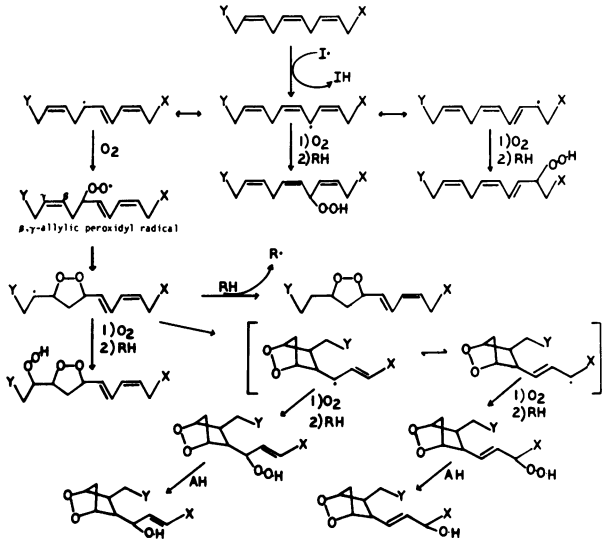

FIGURE 4. Scheme of peroxidation of unsaturated fatty acids to account for mono- and bicyclic peroxides. A β,γ-allylic peroxide formed from trienoic and polyenoic fatty acids is cyclized to either monocyclic peroxides or bicyclic analogs of PGH_2.

dation of unsaturated fatty acids (*15*). Both monocyclic and bicyclic peroxides, which are similar to the Nugteren-Sammuelsson mechanism proposed for prostaglandin cyclo-oxygenase, could be formed. Bicyclic peroxides could resemble the key prostaglandin intermediary, PGH_2/G_2, which is converted to thromboxane A_2, prostacyclin, or prostaglandins E_2, $F_{2\alpha}$, or D_2.

The biopotency of O_3-peroxidized arachidonic acid has been measured by using rabbit aortic strips, rat fundus strips, and human platelets (*16*). Biopotent peroxides, which contract aortic strips with nearly the potency of naturally occurring PGH_2, are formed in the absence of vitamin E. Human platelets are also aggregated by these peroxides at concentrations in the nanomolar range. Hydroperoxides of fatty acids are about 1,000–10,000 times less potent. Addition of 1 mole of vitamin E to 10^3 moles of arachidonic acid reduced the biopotency of the peroxides formed, but not the total peroxides formed. Vitamin E, because of its chemical structure and preferred molecular conformation, could associate with arachidonic acid in such a manner as to favor the formation of hydroperoxides over cyclic peroxides. By donation of its phenolic hydrogen to the β,γ-allylic peroxyl radical, a hydroperoxide would be formed in place of a cyclic peroxide. In support of this hypothesis, an examination of the trienoic and higher fatty acid composition of the mouse lungs showed that the different groups varied by only 2.53 weight %, explaining the similar LT_{50} despite different PI.

An Enzymatic Hydroperoxide Decomposition System

A mechanism exists for the decomposition of hydroperoxides formed in the lung and other tissues. Glutathione peroxidase coupled with glutathione reductase and glucose-6-phosphate dehydrogenase has been proposed for the removal of hydroperoxides formed in tissues (*17*) [Eq. (14)].

Continuous long-term exposure to O_3 has been reported to induce higher levels of the glutathione peroxidase system in the lungs of rats (*17*). NO_2 exposure, on the other hand, does not appear to produce such an induction. We have not found increased glutathione peroxidase levels in mice exposed to 3.4 ± 0.1 ppm NO_2 or in guinea pigs exposed to 1.0 ppm NO_2 as long as 3 months (*18*). In guinea pigs, long-term NO_2 exposure actually results in decreased RBC levels of glutathione peroxidase. A complicating factor is the dependence of glutathione peroxidase levels on vitamin E as shown in Table 7. Fortification of diets with vitamin E decreased both lung and RBC glutathione peroxidase, but NO_2 exposure had no effect.

Conclusion

The toxicity of O_3 and NO_2 is significantly affected by at least one dietary parameter, vitamin E intake. The free-radical-scavenging properties of vitamin E suggest that the pulmonary toxicity of these two air pollutants is mediated through a free radical mechanism. While some effects of NO_2 and O_3 support the hypothesis that fatty acid peroxidation is a major consequence of their inhalation, simple peroxidative effects on cell membranes do not appear adequate to explain the toxicity. The degree of unsaturation of lung fatty acids is not directly correlated with susceptibility to either gas. Rather,

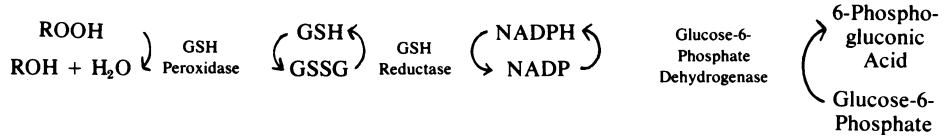

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Table 7. Effect of exposure to 3.4 ± 0.1 ppm NO_2 for 8 hr/day for 24 days on RBC and lung glutathione peroxidase of mice.[a]

Tissue	Glutathione peroxidase activity[b] 0 Vitamin E	105 mg/kg
Lung		
NO_2 exposed	346 ± 11[c]	275 ± 14[d]
Air exposed	325 ± 8	253 ± 14[d]
Red blood cell		
NO_2 exposed	105 ± 6	85 ± 6[e]
Air exposed	120 ± 7	96 ± 5[e]

[a] Mice were fed the high PUFA corn oil diet.
[b] GSH-Px activity is expressed in milli units/mg protein, where 1 unit is equivalent to 1 μmole GSH oxidized/min.
[c] Mean ± SE of four mice per group.
[d] Lung GSH-Px activity was significantly lowered ($p < 0.01$) in mice receiving high supplemental levels of vitamin E.
[e] RBC GSH-Px activity was significantly lowered ($p < 0.05$) in mice receiving high supplemental levels of vitamin E.

the correlation is best with the total trienoic and higher polyenoic fatty acid contents. Arachidonic acid pools within the lung are increased by both oxidants, suggesting a potential role for prostaglandins in the toxicity. Pulmonary edema and inflammation may place higher demands on the prostaglandin system, accounting for this increase. Alternatively, the increased levels of arachidonic acid may represent the specific peroxidation of this PUFA and consequent increased turnover. The products of peroxidation of specific fatty acids may have biological activity producing prostaglandin-like effects. Ozone peroxidation of arachidonic acid produces cyclic peroxides which are almost as potent as the naturally occurring prostaglandin PGH_2. The peroxides are not activated to thromboxane A_2 by platelet thromboxane synthetase and may contain mixtures of many stereoisomers. In the lung, cyclic peroxides could be formed from phospholipids and circulated distally to produce adverse effects.

Vitamin E appears to be the only naturally occurring defense mechanism against O_3 or NO_2 toxicity. The glutathione peroxidase system is induced by O_3 and not by NO_2 and may be inhibited by long-term exposure to NO_2. Glutathione peroxidase levels in both lung and RBCs are suppressed by dietary supplementation with vitamin E. Perhaps the inverse relationship between vitamin E and glutathione peroxidase reflects a lower tissue concentration of peroxides and hence a smaller requirement for enzymatic decomposition with increasing levels of vitamin E. The lung possesses a very active glutathione peroxidase system which can decompose peroxides added to the circulation in blood free perfused lungs (Holden and Menzel, unpublished observations).

The question of the dose-response relationship between dietary vitamin E and O_3 and NO_2 toxicity is only partially resolved. Clear-cut protection against O_3 mortality occurs only at dietary levels of 100 mg vitamin E/kg of diet. These levels are effective against O_3 inhalation of up to 1 ppm. Beyond this concentration, 1.6 ppm or greater, the protective effect of vitamin E is overcome by O_3. Ozone is a highly reactive compound and direct reaction with tissue constituents such as pyridine nucleotides or thiols may contribute to the toxicity. Glutathione peroxidase function may be inhibited by a lack of reduced glutathione. Perfusion of the lung with high concentrations of peroxides over 1 μM removes almost all of the glutathione content of the lung and produces severe damage (Holden and Menzel, unpublished results).

When mice are exposed to subacute concentrations of either NO_2 or O_3, lung edema occurs, despite the provision of vitamin E at levels as high as 100 mg vitamin E/kg of diet. Such a dietary intake corresponds in the mouse to roughly 3 I.U. vitamin E/kg body weight/day, which is similar to the present human intake of 2.4 I.U. vitamin E/kg body weight/day. Levels as low as 0.3 I.U. vitamin E/kg body weight/day are effective in reducing lung edema in mice exposed to 13 ppm NO_2. The maximum O_3 and NO_2 concentrations found in urban air are about 0.3 ppm for each. The present dietary intake of vitamin E is minimally adequate at 3 times the average ambient concentration of O_3 and 40 times the average ambient concentration of NO_2. At any O_3 or NO_2 exposure level, the index of lung edema used here is still elevated over control clean air-exposed animals, suggesting that the consumption of higher levels of vitamin E by man may be required for further protection.

The general phenomenon of lipid peroxidation following exposure to a toxicant which can be converted to a free radical source suggests that the present dietary intake of vitamin E may also be inadequate to protect man. Plaa and Witschi (*19*) have reviewed other chemicals and drugs which promote lipid peroxidation and have suggested that peroxidation is a more general toxicant response than previously supposed. The present proposal that oxidant toxicity is mediated through the production of biopotent products formed during peroxidation would apply equally well to any other toxicant which can initiate peroxidation.

Oxidant toxicity may be expressed in chronic diseases, such as emphysema for NO_2 or O_3 or liver necrosis for halogenated solvents. The similarity of the toxicant-produced peroxides to natural peroxides produced by the prostaglandin system points to potential interactions with other diseases

known to have aberrant prostaglandin function. Particularly intriguing possibilities are interactions with asthma and cardiovascular disease. Asthmatics are particularly sensitive to prostaglandins, leading to the concept that they have an imbalance between prostaglandins of opposing effects. Asthmatics may be especially sensitive to NO_2 exposure. Arterial thrombosis has been suggested as the primary cause of plaque formation and as a precursor of cardiovascular disease. A key step in this process may be the prostaglandin system. Prostaglandin endoperoxides, PGG_2/H_2, are converted to thromboxane A_2 by platelets and to prostacyclin by arterial endothelial cells. Thromboxane A_2 promotes platelet adhesion and thrombosis while prostacyclin disaggregates platelets and reduces adhesion. Prostacyclin synthetase is inhibited by 15-hydroperoxyl fatty acids. Non-specific peroxides produced by toxicants could inhibit the activity of this enzyme. Interestingly, cigarette smoke, a common underlying cause of cardiovascular disease, contains about 1,000 ppm NO, a small portion of which is continuously being converted to NO_2 by reaction with other constituents.

These experiments and speculations on the role of one dietary component, vitamin E, in the response to environmental toxicants also indicates the need to reevaluate traditional criteria for vitamin and nutrient function. Biochemical criteria, rather than frank signs of deficiency, should be used to evaluate the present need for nutrients. Stresses placed on man through his changing environment may require higher levels of a specific nutrient than previously supposed on the basis of more classical criteria. Such a view is particularly attractive to the concept of preventive medicine and the maintenance of a high quality of life through regulation of environmental hazards.

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REFERENCES

1. Cortesti, R., and Privett, O. S. Toxicity of fatty acid ozonides and peroxides. Lipids 7: 715 (1972).
2. Menzel, D. B., Slaughter, R. J., Bryant, A. M., and Jauregui, H. O. Heinz bodies formed in erythrocytes by fatty acid ozonides and ozone. Arch. Environ. Health. 30: 296 (1975).
3. Menzel, D. B., Roehm, J. N., and Lee, S. D. Vitamin E: the biological and environmental antioxidant. J. Agr. Food Chem. 20: 481 (1972).
4. Fletcher, B. L., and Tappel, A. L. Protective effects of dietary α-tocopherol in rats exposed to toxic levels of O_3 and NO_2. Environ. Res. 6: 165 (1973).
5. Mustafa, M. G. Influence of dietary vitamin E on lung cellular sensitivity to ozone in rats. Nutr. Rep. Intern. 11: 473 (1975).
6. Donovan, D. H., Williams, S. J., Charles, J. M., and Menzel, D. B. Ozone toxicity: effect of dietary vitamin E and polyunsaturated fatty acids. Toxicol. Letters. 1: 135 (1977).
7. Bieri, J. G., and Poukka, R. K. H. *In vitro* hemolysis as related to rat erythrocyte content of α-tocopherol and polyunsaturated fatty acids. J. Nutr. 100: 557 (1970).
8. Goldstein, B. D., Buckley, R. D., Cardenas, R., and Balchum, O. J. Ozone and vitamin E. Science 169: 605 (1970).
9. Menzel, D. B. The role of free radicals in the toxicity of oxidizing air pollutants (nitrogen oxides and ozone). In: Free Radicals in Biology, Vol. 2, W. A. Pryor, Ed., Academic Press, New York, 1976.
10. Alfin-Slater, R. B., Shimma, Y., Hansen, H., Wells, P., Aftergood, L., and Melnick, D. Dietary fat composition and tocopherol requirement. III. Quantitative studies on the relationship between dietary linoleate and vitamin E. J. Am. Oil Chemists' Soc. 49: 395 (1972).
11. Witting, L. A., and Horwitt, M. K. Effect of degree of fatty acid unsaturation in tocopherol deficiency-induced creatinuria. J. Nutr. 82: 19 (1964).
12. Mead, J. F. Free radical mechanisms of lipid damage and consequences for cellular membranes. In: Free Radicals in Biology, Vol. 1, W. A. Pryor, Ed., Academic Press, New York, 1976.
13. Hadley, J. G., Gardner, D. E., Coffin, D. L., and Menzel, D. B. Enhanced binding of autologous red cells to the macrophage plasma membrane as a sensitive indicator of pollutant damage. In: Pulmonary Macrophage and Epithelial Cells. C. L. Sanders, R. P. Schneider, G. E. Dagle, and H. A. Ragan, Eds., Technical Information Center, E. R. D. A., Washington, D. C., 1977.
14. Menzel, D. B. The role of vitamin E in environmental toxicity. In: Vitamin E. L. J. Machlin, Ed., Marcel Dekker, New York, 1979.
15. Pryor, W. A., Stanley, J. P., and Blair, E. Autoxidation of polyunsaturated fatty acids. II. A suggested mechanism for the formation of TBA-reactive materials from prostaglandin-like endoperoxides. Lipids 11: 370 (1976).
16. Roycroft, J. H., Gunter, W. B., and Menzel, D. B. Ozone toxicity: hormone-like oxidation products from arachidonic acid by ozone catalyzed autoxidation. Toxicol. Letters 1: 75 (1977).
17. Chow, C. K., and Tappel, A. L. An enzymatic mechanism against lipid peroxidation damage to lungs of ozone-exposed rats. Lipids 7: 518 (1972).
18. Menzel, D. B., Abou-Donia, M. B., Roe, C. R., Ehrlich, R. E., Gardner, D. E., and Coffin, D. L. Biochemical indices of nitrogen dioxide intoxication of guinea pigs following low level long-term exposure. In: International Conference on Photochemical Oxidant Pollution and Its Control Proceedings. Vol. II, EPA 600/3-77-001b, Washington, D. C., 1977.
19. Plaa, G. L., and Witschi, H. Chemicals, drugs and lipid peroxidation. Ann. Rev. Pharmacol. Toxicol. 16: 125 (1976).